
SUPPORT FOR AN EXPANDED *SOLMS-LAUBACHIA* (BRASSICACEAE): EVIDENCE FROM SEQUENCES OF CHLOROPLAST AND NUCLEAR GENES^{1,2}

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ABSTRACT

Sequences of the plastid maturase (*matK*) and nuclear chalcone synthase (*Chs*) were analyzed separately and in combination to assess phylogenetic relationships of *Solms-laubachia* Muschl. (all nine known species and two undescribed ones) to the genera *Baimashania* Al-Shehbaz, *Parrya* R. Br., *Desideria* Pamp., *Leiospora* (C. A. Mey.) Dvořák, *Christolea* Cambess., and *Phaeonychium* O. E. Schulz (Brassicaceae). *Baimashania* is clustered with *Aubrieta deltoidea* (L.) DC. and *Arabis blepharophylla* Hook. & Arn. *Solms-laubachia*, *Desideria*, *Leiospora*, *Christolea*, and *Phaeonychium* are more closely related to *Matthiola* R. Br. than to *Parrya*, and they form a well-supported clade. Within this clade, *Leiospora* is sister to a subclade containing the sister groups *Christolea* and *Solms-laubachia* s.l. The *Solms-laubachia* s.l. group contains *Solms-laubachia*, *Desideria*, and a species of *Phaeonychium*. Neither *Solms-laubachia* nor *Desideria* is monophyletic, whereas *Phaeonychium jafrii* Al-Shehbaz is embedded within the group. Therefore, our results suggest that *Solms-laubachia* be expanded to include *Desideria* and *P. jafrii*. Nevertheless, more species of *Desideria*, *Parrya*, and *Phaeonychium* are needed to further test our findings.

Key words: Brassicaceae, *Christolea*, *Chs*, *Desideria*, *Leiospora*, *matK*, *Parrya*, *Phaeonychium*, phylogeny, *Solms-laubachia*.

The Brassicaceae (Cruciferae) consist of approximately 340 genera and 3350 species (Al-Shehbaz, 1984; Appel & Al-Shehbaz, 2003). They are a monophyletic family characterized by a number of synapomorphies, including flower structure (e.g., four sepals, four petals, often six tetradynamous stamens) and often a dehiscent capsule derived from a bicarpellate pistil with a septum dividing its ovary into two locules. Many plants of this family are common vegetables (e.g., cabbages, broccoli, cauliflower, Brussels sprouts, radishes, turnips, watercress). *Arabidopsis thaliana* (L.) Heynh., the model organism in plant experimental biology, belongs to the Brassicaceae.

Before the advent of molecular systematics, classification systems of the family (e.g., Hayek, 1911; Schulz, 1936; Janchen, 1942; Al-Shehbaz, 1984) were based almost exclusively on morphology. Over the past decade, molecular studies have greatly contributed to a better understanding of phylogenetic

relationships within the Brassicaceae. Such studies show that many characters that have been commonly used to define tribal and subtribal divisions in the family are homoplasious. As a result, many tribes as traditionally recognized (e.g., Arabideae DC., Lepidieae DC., Euclidieae DC., Sisymbrieae DC.) are highly artificial (Koch et al., 2003; Al-Shehbaz et al., 2006). Generic limits within the family have also been a major source of controversy (Al-Shehbaz, 1984; Appel & Al-Shehbaz, 2003; Al-Shehbaz et al., 2006). However, molecular data have shed enormous light on the monophyly of various genera. For example, results from the nuclear ribosomal DNA internal transcribed spacer (nrDNA ITS) sequence data show that *Arabis* L. and *Arabidopsis* Heynh., as traditionally delimited, are polyphyletic because they included purportedly unrelated species that had been assigned to many other genera (Koch et al., 1999; Al-Shehbaz et al., 1999; Al-Shehbaz, 2003; O'Kane & Al-Shehbaz, 2003; Al-

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Shehbaz, 2005). Warwick et al. (2002, 2004) have shown that generic limits of *Sisymbrium* L. and *Smelowskia* C. A. Mey. need reconsideration.

Another taxonomically difficult group involves several Himalayan and Central Asian genera, including *Solms-laubachia* Muschl. (9 spp.), *Desideria* Pamp. (12 spp.), *Leiospora* (C. A. Mey.) Dvořák (6 spp.), *Christolea* Cambess. (2 spp.), *Parrya* R. Br. (25 spp.), and *Phaeonychium* O. E. Schulz (7 spp.) (Appel & Al-Shehbaz, 2003). These genera occupy similar habitats (alpine scree slopes and limestone crevices) and exhibit overlapping morphological characters (e.g., perennial habit, latiseptate or terete fruits, well-defined basal rosettes, non-saccate sepals). Consequently, the same species have often been placed in different genera. For example, *Desideria pumila* (Kurz) Al-Shehbaz was originally described as *Parrya* (Kurz, 1872) and later transferred to *Christolea* (Jafri, 1955), *Vvedenskeyella* Botsch. (Botschantsev, 1955), or *Desideria* (Al-Shehbaz, 2001).

Parrya is most highly diversified in central Asia but has three species occurring in Arctic and subarctic Asia and North America (Appel & Al-Shehbaz, 2003). Four of the nine known species of *Solms-laubachia*, three of the 12 of *Desideria*, and all six of *Leiospora* were originally described as species of *Parrya* (Schulz, 1936). Recent morphological (Al-Shehbaz, 2001; Al-Shehbaz & Yang, 2001) and cytological (Yue et al., 2003, 2004) studies have indicated that while *Desideria*, *Solms-laubachia*, *Christolea*, and *Leiospora* are closely related to one another, together they are distinct from *Parrya*. Nevertheless, none of these genera have been included in phylogenetic analyses based on molecular data (Koch et al., 2003).

Baimashania Al-Shehbaz (2 spp.) is a recently described genus from China based on specimens that have been misidentified as species of *Solms-laubachia* or *Leiospora* (Al-Shehbaz, 2000b).

The objective of this study was to infer phylogenetic relationships of *Solms-laubachia* to the presumably related genera *Baimashania*, *Christolea*, *Desideria*, *Leiospora*, *Parrya*, and *Phaeonychium* based on sequences of the chloroplast gene *matK* (maturase) and the nuclear gene *Chs* (chalcone synthase). These two markers have been used successfully in resolving generic relationships within Brassicaceae (e.g., Koch et al., 2000, 2001).

MATERIALS AND METHODS

PLANT MATERIAL

Fifty samples were used in this study, including 11 species of *Solms-laubachia* (nine in total plus two undescribed species), four of *Desideria* (of 12), two of

Leiospora (of six), and one each of *Baimashania* (of two), *Phaeonychium* (of seven), *Christolea* (of two), and *Parrya* (of 25). The remaining samples represent 24 genera of Brassicaceae. This broad sampling of the family is necessary because none of these genera mentioned above have been included in previous phylogenetic analyses and it is unclear whether they form a clade and what taxa are their outgroups. As in Koch et al. (2001), we used *Aethionema grandiflorum* Boiss. & Hohen. as the outgroup because *Aethionema* R. Br. is sister to the rest of Brassicaceae (Zunk et al., 1996; Galloway et al., 1998). In their recently proposed tribal classification of the family, Al-Shehbaz et al. (2006) placed *Parrya* with *Matthiola* R. Br. in the tribe Anchonieae DC., *Baimashania* in the Arabideae, and *Solms-laubachia*, *Desideria*, and *Leiospora* in the Euclidieae.

DNA EXTRACTION, PCR, AND SEQUENCING

Total genomic DNAs were extracted from silica gel-dried leaves using a Qiagen DNeasy Plant Mini Kit following the manufacturer's protocol (Santa Clarita, California). Genomic DNAs of three accessions of *Desideria* and one each of *Leiospora* and *Solms-laubachia* were kindly provided by Jason R. Grant (Université de Neuchâtel, Switzerland).

Plastid *matK*

Double-stranded DNAs of *matK* were amplified using primers *trnK*-710F* and *trnK*-2R* originally designed by Johnson and Soltis (1994) and slightly modified later by Koch et al. (2001). A 50- μ L polymerase chain reaction (PCR) included 50–100 ng DNA, 5 μ L of 10 $\times$ *Taq* polymerase buffer, 4 μ L of dNTP (2.5 mM), 4 μ L of MgCl₂ (25 mM), 2 μ L of each primer (10 μ M), 0.5 μ L of *Taq* polymerase (5 units/ μ L), and 29.5–31.5 μ L of sterilized water, depending on the amount of DNA used. The PCR profile used to amplify the *matK* region included 34 cycles (94°C for 1 minute 30 seconds, 48–52°C for 2 minutes, 72°C for 3 minutes) and a final extension at 72°C for 15 min. PCR products were gel-purified using a Qiagen Gel Purification Kit, following the manufacturer's protocol. Both strands were sequenced using the ABI BigDye Florescent Terminator Chemistry (Applied Biosystems, Foster City, California) and the following primers: *trnK*-710F*, *trnK*-2R*, *matK*-1F, *matK*-312F, *matK*-495F, *matK*-495R, *matK*-1089R, and *matK*-1089R (Koch et al., 2001).

Nuclear *Chs*

PCR amplification of the entire gene, including the promoter region, was performed using the primers

Chs-For1 and *Chs*-Rev 5 (Koch et al., 1999). Components of the PCR reaction were the same as those described for the *matK* gene, and the PCR program included a 5-minute denaturation at 95°C and 35 cycles, followed by an additional 15-minute extension at 72°C. Each cycle consisted of 1 minute of denaturing at 95°C, 1 minute of annealing at 55°C, and a 2-minute extension at 72°C. All of the PCR amplifications resulted in a single predominant band on an agarose gel. PCR products were gel-purified using a Qiagen Gel Purification Kit, following the manufacturer's protocol, and then were cloned into the pGEM-T cloning vector (Promega, Madison, Wisconsin). To detect possible allelic variation, two clones from two independent PCR reactions were sequenced respectively for each sample. Sequencing primers included vector primers T7 and Sp6, and Koch et al.'s (1999) primers (*Chs*-FOR2, *Chs*-REV2, *Chs*-REV3, *Chs*-FOR4, and *Chs*-REV4).

Sequences were analyzed using an Automated ABI Genetic Analyzer 3100, and were then edited using Sequencher (version 4.0, Gene Codes Corp., Inc., Ann Arbor, Michigan). The edited sequences were then aligned manually.

PHYLOGENETIC ANALYSES

Phylogenetic analyses were performed for the data sets individually and in combination using maximum parsimony (Farris et al., 1970; Fitch, 1971) and Bayesian methods (Rannala & Yang, 1996). The heuristic searches for maximum parsimony analysis were conducted using PAUP*4.0 (Swofford, 2002) with the following conditions: random sequence addition with 1000 replicates and 10 trees held at each step, tree bisection-reconnection branch swapping, and MULPARS on and the steepest descent off. Character state changes were unordered and equally weighted in the analysis, and gaps were treated as missing data. Bootstrap analyses of 1000 replicates were performed to estimate the support for individual clades using heuristic tree search options as in the maximum parsimony analyses. To further assess relative support for the individual clades, a decay analysis (Sorenson, 1996) was also used. The best evolutionary model of DNA substitutions for the Bayesian analysis was determined using Modeltest version 3.06 (Posada & Crandall, 1998). Bayesian analyses (Rannala & Yang, 1996; Mau et al., 1999) were carried out using MrBayes version 3.0b3 (Huelsenbeck & Ronquist, 2001, available at <<http://mrbayes.csit.fsu.edu>>) with the model parameters as estimated in Modeltest. Bayesian analysis was started from random trees and employed four Markov chain Monte Carlo runs, monitored over $2 \times$

10^6 generations, sampling trees every 100 generations. Runs were repeated twice to confirm results. The resulting log likelihood and number of generations were plotted to determine the point after which the log likelihoods had stabilized. After discarding the trees saved prior to this point as burn-in, the remaining trees were imported into PAUP*, and a 50% majority-rule tree was produced to obtain posterior probabilities of the clades (Huelsenbeck & Ronquist, 2001). Internodes with posterior probabilities $\geq 95\%$ were considered statistically significant.

The incongruence length difference (ILD) test (Farris et al., 1994, as implemented in PAUP*) was used to assess potential conflicts between the phylogenetic signals from the two different DNA fragments. For the test, 100 replicates were analyzed with a heuristic search, each with 10 random sequence addition replicates. In addition, we compared *matK* and *Chs* trees to examine whether there were well-supported ($> 70\%$ bootstrap support), but conflicting clades.

RESULTS

SEQUENCE CHARACTERISTICS

Twenty-five *matK* and 24 *Chs* sequences were newly gathered in this study. An additional 26 *matK* and *Chs* sequences were obtained from GenBank and included in this study (Table 1).

The amplified segment also included a 300-bp fragment at the 3' end of the *trnK* region. However, the alignment of this region across Brassicaceae was ambiguous, and thus our analyses did not include sequences of the segment. The sequences of the *matK* gene were aligned readily by hand, resulting in a data set of 1536 bp, of which 530 sites were variable and 237 sites (15.43%) were potentially parsimony informative. The sequence divergence ranged from 6.02%–8.27% between the outgroup and ingroup and from 0.067%–7.93% within the ingroup.

Limits of the introns and promoter regions of the *Chs* gene were determined by comparison with published sequences (Koch et al., 1999). These regions could not be aligned unambiguously across Brassicaceae and thus were removed from phylogenetic analyses. However, exon sequences were aligned readily by hand, and the alignment resulted in a data set of 1174 bp, of which 511 were variable sites and 364 sites (31%) were potentially parsimony informative. The sequence divergence ranged from 19.3%–23.6% between the outgroup and ingroup and from 0.34%–17.9% within the ingroup.

Results of the ILD test showed that our *matK* and *Chs* data sets were not significantly different ($P =$

Table 1. Vouchers and sources of species used in this study. An asterisk (*) indicates that sequences were obtained from GenBank.

Taxon	Voucher	Source	matK	Chs
<i>Aethionema grandiflorum</i> Boiss. & Hohen.			AF144354*	AF112082*
<i>Alliaria petiolata</i> (M. Beib.) Cavara & Grande			AF144363*	AF144537*
<i>Arabidopsis thaliana</i> (L.) Heynh.			AF144348*	AF112086*
<i>Arabis blepharophylla</i> Hook. & Arn.			AF144353*	AF112087*
<i>Arabis divaricarpa</i> A. Nelson			AF144351*	AF112090*
<i>Aubrieta deltoidea</i> (L.) DC.			AF144352*	AF112109*
<i>Baimashania pulvinata</i> Al-Shehbaz	Yue 0222 (KUN)	China, Yunnan, Deqing	DQ409251	DQ409227
<i>Barbarea vulgaris</i> R. Rr.			AF144330*	AF112108*
<i>Capsella rubella</i> Reuter			AF144334*	AF112106*
<i>Cardamine amara</i> L.			AF144337*	AF112085*
<i>Cardamine penzesii</i> Ančev & Marhold			AF144364*	AF144538*
<i>Cardamine rivularis</i> Schur			AF144365*	AF144539*
<i>Christolea crassifolia</i> Cambess.	B. Bartholomew et al. 9499 (MO)	China, Xinjiang	DQ409256	DQ409232
<i>Cochlearia danica</i> L.			AF174531*	AF144532*
<i>Crucihimalaya himalaica</i> (Edgew.) Al-Shehbaz, O’Kane & R. A. Price			AF144356*	AF144531*
<i>Desideria baiogoinensis</i> (K. C. Kuan & Z. X. An) Al-Shehbaz	Yue 0246 (KUN)	China, Tibet, Mozhugongka	DQ409252	DQ409228
<i>Desideria himalayensis</i> (Cambess) Al-Shehbaz	McBenth 1486 (E)	Nepal	DQ409266	DQ409242
<i>Desideria linearis</i> (N. Busch) Al-Shehbaz	B. Bartholomew et al. 9549 (MO)	China, Xinjiang	DQ409254	DQ409230
<i>Desideria stewartii</i> (Anderson) Al-Shehbaz	McBeath 2105 (E)	India	DQ409265	DQ409241
<i>Erysimum handel-mazzettii</i> Polatschek	Yue 0369 (KUN)	China, Yunnan, Zhongdian	DQ409262	DQ409238
<i>Fourraea alpina</i> (L.) Greuter & Burdet			AF144335*	AF112102*
<i>Halimolobus perplexa</i> (Henderson) Rollins var. <i>perplexa</i>			AF144346*	AF112094*
<i>Ionopsidium abulense</i> (Pau.) Rothm.			AF144368*	AF144542*
<i>Leiospora exscapa</i> (C. A. Mey.) Dvořák	Murray et al. 457 (ALA)	Russia	DQ409263	DQ409239
<i>Leiospora pamirica</i> (Botsch. & Vved.) Botsch. & Pachom.	B. Bartholomew et al. 9790 (MO)	China, Xinjiang	DQ409255	DQ409231
<i>Lepidium campestre</i> (L.) R. Br.			AF144359*	AF144534*
<i>Matthiola incana</i> (L.) R. Br.			AF144361*	AJ427536*
<i>Microthlaspi perfoliatum</i> (L.) F. K. Meyer			AF144362*	AF144536*
<i>Olimarabidopsis cabulica</i> (Hook. f. & Thompson) Al-Shehbaz, O’Kane & R. A. Price			AF144358*	AF144533*
<i>Olimarabidopsis pumila</i> (Stephan) Al-Shehbaz, O’Kane & R. A. Price			AF144345*	AF112092*
<i>Parrya nudicaulis</i> (L.) Regel	Yue 0244 (KUN)	China, Tibet, Lhasa	DQ409253	DQ409229
<i>Phaeonychium jafrii</i> Al-Shehbaz	Yue 0233 (KUN)	China, Tibet, Lhasa	DQ409261	DQ409237
<i>Rorippa amphibia</i> (L.) Besser			AF174530*	AF144530*
<i>Sinapis alba</i> L.			X04826*	X16437*
<i>Sisymbrium irio</i> L.			AF144366*	AF144541*
<i>Solms-laubachia eurycarpa</i> (Maxim.) Botsch., 1	SAB Yushu Exped. 2178 (E)	China, Qinghai, Yushu	DQ409264	DQ409240
<i>Solms-laubachia eurycarpa</i> (Maxim.) Botsch., 2	Yue 0158 (KUN)	China, Yunnan, Deqing	DQ409243	DQ409219
<i>Solms-laubachia lanata</i> Botsch.	Yue 0234 (KUN)	China, Tibet, Lhasa	DQ409246	DQ409222
<i>Solms-laubachia linearifolia</i> (W. W. Sm.) O. E. Schulz	Yue 0157 (KUN)	China, Yunnan, Deqing	DQ409249	DQ409225
<i>Solms-laubachia minor</i> Hand.-Mazz.	Yue 0379 (KUN)	China, Sichuan, Yanyuan	DQ409257	DQ409233

Table 1. Continued.

Taxon	Voucher	Source	matK	Chs
<i>Solms-laubachia platycarpa</i> (Hook. f. & Thomson) Botsch.	Yue 0238 (KUN)	China, Tibet, Dangxion	DQ409245	DQ409221
<i>Solms-laubachia pulcherrima</i> Muschl.	Yue 0153 (KUN)	China, Yunnan, Lijian	DQ409247	DQ409223
<i>Solms-laubachia retropilosa</i> Botsch.	Yue 0248 (KUN)	China, Sichuan, Kangding	DQ409248	DQ409224
<i>Solms-laubachia</i> sp. indet. 1	D. E. Boufford et al. 30727 (HUH)	China, Sichuan, Xiangcheng	DQ409258	DQ409234
<i>Solms-laubachia</i> sp. indet. 2	D. E. Boufford et al. 31975 (HUH)	China, Tibet, Riwoqe	DQ409260	DQ409236
<i>Solms-laubachia xerophyta</i> (W. W. Sm.) Comber, 1	Yue 0250 (KUN)	China, Sichuan, Daocheng	DQ409244	DQ409220
<i>Solms-laubachia xerophyta</i> (W. W. Sm.) Comber, 2	Yue 0251 (KUN)	China, Yunnan, Zhongdian	DQ409259	DQ409235
<i>Solms-laubachia zhongdianensis</i> J. P. Yue, Al-Shehbaz & H. Sun	Yue 0156 (KUN)	China, Yunnan, Zhongdian	DQ409250	DQ409226
<i>Thlaspi arvense</i> L.			AF144360*	AF144535*
<i>Turritis glabra</i> L.			AF144333*	AF112091*

0.51). Furthermore, maximum parsimony and Bayesian analyses based on the two individual data sets resulted in several well-supported monophyletic groups, and there were no well-supported (bootstrap support > 70% and posterior probability > 95%) but incongruent clades (trees not shown). Therefore, results of phylogenetic analyses based on the combined data set are presented below.

PHYLOGENETIC RELATIONSHIPS

The combined data set of *matK* and *Chs* had 50 taxa and 2710 sites, of which 1669 (61.6%) were invariable, 440 (16.2%) were unique nucleotide changes, and 601 (22.2%) were parsimony informative. The sequence divergence ranged from 6.02%–8.27% between the outgroup and ingroup and 0.067%–7.93% within the ingroup.

Parsimony analyses yielded six trees of 2889 steps; the strict consensus is shown in Figure 1 (consistency index (CI) = 0.50, retention index (RI) = 0.69, and rescaled consistency index (RC) = 0.35). The relationships of the taxa whose *matK* and *Chs* sequences were obtained from the GenBank (cf. Table 1) were consistent with those of Koch et al. (2001). To these, we included our samples of *Baimashania pulvinata* Al-Shehbaz and *Erysimum handel-mazzettii* Polatschek. The former species clustered with *Aubrieta deltoidea* (L.) DC. and *Arabis blepharophylla* Hook. & Arn. (Clade A) and was remotely related to both *Solms-laubachia* and *Leiospora*, whereas the latter was positioned in a clade

including *Arabidopsis*, *Turritis* L., *Olimarabidopsis* Al-Shehbaz, O’Kane & R. A. Price, *Capsella* Medik., *Arabis* L., *Halimolobus* Tausch, and *Crucihimalaya* Al-Shehbaz, O’Kane & R. A. Price.

Parrya nudicaulis (L.) Regel was strongly supported as sister to the clade (Clade B, Fig. 1) containing *Matthiola incana* (L.) R. Br., *Leiospora*, *Christolea*, *Solms-laubachia*, *Desideria*, and *Phaeonychium jafrii* Al-Shehbaz. *Matthiola* was sister to the clade containing the latter five genera (Clade C, Fig. 1). In Clade C, two accessions of *Leiospora* formed a well-supported group, occupying a sister position to other remaining taxa that formed Clade D (Fig. 1). Species of *Solms-laubachia*, *Desideria*, and *P. jafrii* formed a clade (Fig. 1, Clade E), with *C. crassifolia* Cambess. as a sister group. Within Clade E, there were three subclades, the first of which (Clade F) included *D. linearis* (N. Busch) Al-Shehbaz, *D. stewartii* (Anderson) Al-Shehbaz, and *D. himalayensis* (Cambess.) Al-Shehbaz. The second had *P. jafrii* alone and was sister to the third clade (Clade G), which contained *D. baiogoinensis* (K. C. Kuan & Z. X. An) Al-Shehbaz and all species of *Solms-laubachia*. While interspecific relationships of *Solms-laubachia* were weakly or moderately supported, *S. lanata* Botsch. and *D. baiogoinensis* clustered together with strong support (bootstrap = 94%). Clade G was a trichotomy including *D. baiogoinensis* + *S. lanata*, *S. platycarpa* (Hook. f. & Thomson) Botsch., and Clade H. Within this clade, *S. retropilosa* Botsch. and *S. linearifolia* (W. W. Sm.) O. E. Schulz formed a moderately supported clade (bootstrap = 74%) that was sister to

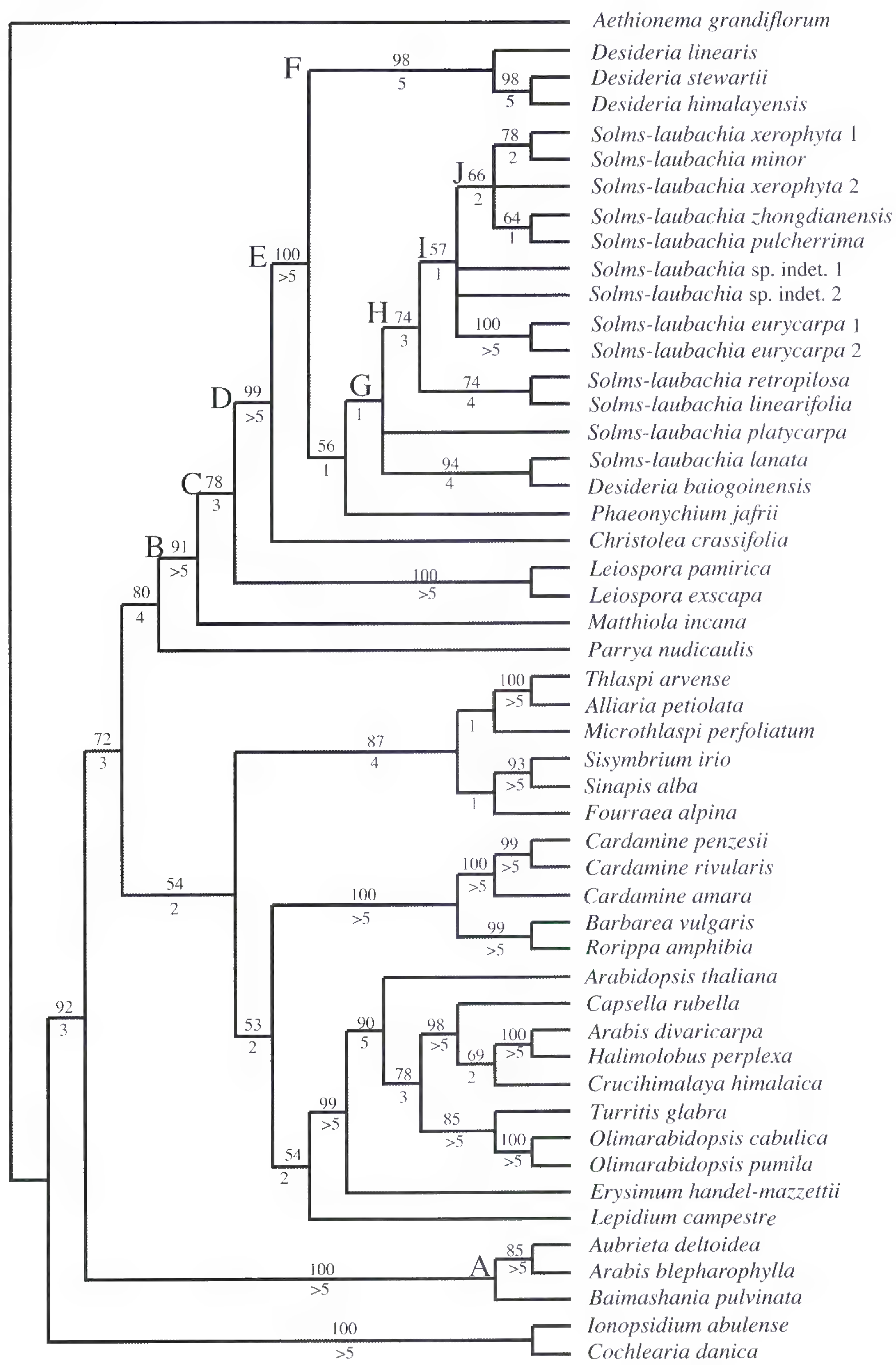


Figure 1. Strict consensus of six parsimonious trees based on the combined data set of *matK* and *Chs* genes. Numbers above and below branches are bootstrap percentages and decay indices, respectively. Letters A–J refer to clades discussed in the text.

a polytomous branch (Clade I, bootstrap = 57%). Clade I contained *S. eurycarpa* (Maxim.) Botsch., two undescribed species (*Solms-laubachia* sp. indet. 1 and 2), and Clade J, which consists of *S. minor* Hand.-Mazz. + one accession of *S. xerophyta* (W. W. Sm.) Comber (bootstrap = 78%), *S. zhongdianensis* J. P. Yue, Al-Shehbaz & H. Sun + *S. pulcherrima* Muschl. (bootstrap = 64%), and the second accession of *S. xerophyta* (W. W. Sm.) Comber.

The best-fit model of evolution for the combined *matK* and *Chs* data set was GTR + I + G, as indicated by the Modeltest. The base frequencies were A = 0.287, C = 0.195, G = 0.19, T = 0.328, and the gamma distribution shape parameter was 0.6244. Bayesian analyses with the estimated parameters produced trees with identical topology to Figure 1, with a few discrepancies in the clade containing species of *Solms-laubachia*, *Desideria baiogoinensis*, and *Phaeonychium jafrii* (Fig. 2). *Phaeonychium jafrii* formed a sister relationship with Clade G in the maximum parsimony tree (Fig. 1), while in the Bayesian tree *S. platycarpa* was sister to the clade consisting of *D. baiogoinensis*, *P. jafrii*, and the remaining species of *Solms-laubachia* (Fig. 2). In the maximum parsimony tree, *S. eurycarpa* and the two undescribed *Solms-laubachia* species formed a polytomy. However, in the Bayesian tree, one undescribed species (*Solms-laubachia* sp. indet. 1) clustered with the two accessions of *S. eurycarpa*, while the other (*Solms-laubachia* sp. indet. 2) was sister to the clade containing *S. eurycarpa*, *S. sp. indet. 1*, *S. xerophyta*, *S. minor*, *S. zhongdianensis*, and *S. pulcherrima*. Nevertheless, the topological differences between the maximum parsimony and Bayesian trees were weakly supported.

DISCUSSION

Schulz (1936) placed *Solms-laubachia* and *Parrya* (including *Leiospora*) with *Matthiola* in the tribe Matthioleae O. E. Schulz, *Christolea* in the tribe Sisymbrieae, and *Desideria* (as a synonym of *Ermania* Cham.) and *Phaeonychium* in the tribe Arabideae. These tribes were defined largely on the basis of silique fruits with incumbent cotyledons and open calyx (Sisymbrieae), accumbent cotyledons and closed calyx (Matthioleae), or accumbent cotyledon and open calyx (Arabideae). However, in the *matK* + *Chs* tree (Fig. 1), *Matthiola* is sister to the clade containing *Christolea*, *Desideria*, *Solms-laubachia*, *Phaeonychium*, and *Leiospora* (bootstrap = 78%), suggesting that these genera are closely related to one another and, together, to *Matthiola*. Our results provide further evidence that most tribes of Schulz (1936) are not monophyletic (Koch et al., 2003; Al-Shehbaz et al., 2006).

Specimens of *Baimashania pulvinata*, a species endemic to northwestern Yunnan, had been misidentified as *Solms-laubachia ciliaris* (Bureau & Franch.) Botsch. (Al-Shehbaz, 2000b). Our sequence data did not place *Baimashania* close to *Solms-laubachia*. Instead, these data support a close relationship of *Baimashania* with *Aubrieta* Adans. and *Arabis*. Therefore, our results support the recognition of *Baimashania* as a separate genus from *Solms-laubachia*, and Al-Shehbaz et al. (2006) assigned these two genera to the tribes Arabideae and Euclidieae, respectively.

In both the maximum parsimony and Bayesian trees (Figs. 1, 2), *Parrya* is sister to the clade (B) containing *Matthiola*, *Leiospora*, *Christolea*, *Desideria*, *Solms-laubachia*, and *Phaeonychium*, indicating that none of the latter five genera is closely related to *Parrya*. This is supported by morphological and cytological evidence (Al-Shehbaz, 2001; Al-Shehbaz & Yang, 2001; Yue et al., 2003, 2004). *Solms-laubachia* differs from *Parrya* in that it has wingless seeds, rounded replum concealed by the strongly angled valve margins, valves apically united with obsolete style and replum, fruits readily detached basally from the pedicel, entire stigmas or with non-decurrent lobes, and eglandular papillae. By contrast, *Parrya* has winged seeds, strongly flattened and readily visible replum, flat and not angled valve margins, valves readily separated from the well-developed styles, fruits persistently united basally with pedicels, stigmas with decurrent and connate lobes, and often glandular papillae (Al-Shehbaz & Yang, 2001). *Solms-laubachia*, *Desideria*, and *Christolea* are more similar to one another in chromosome morphology than they are to *Parrya* (Yue et al., 2003, 2004). Nevertheless, an extensive sampling of *Parrya* is needed to further test the phylogenetic position of the genus.

Solms-laubachia, *Desideria*, and *Phaeonychium jafrii* form a well-supported clade (E, Figs. 1, 2), and their close relationship is supported by the smooth fruit valve (Al-Shehbaz, 2000a, 2001). Together, these three genera form a clade sister to *Christolea*. Several morphological synapomorphies offer support for the relationship, such as the presence of cauline leaves, equal and non-saccate sepals, obsolete or distinct style, non-decurrent stigma lobes, and wingless seeds. By contrast, in *Leiospora*, which is sister to the clade of *Christolea*, *Solms-laubachia*, *Desideria*, and *Phaeonychium jafrii*, cauline leaves are absent, sepals unequal and saccate, style absent, stigma lobes strongly decurrent, and seeds winged (Al-Shehbaz, 2000a, 2001).

Species of *Desideria* form a clade (F, Figs. 1, 2) except for *D. baiogoinensis*, which is embedded with-

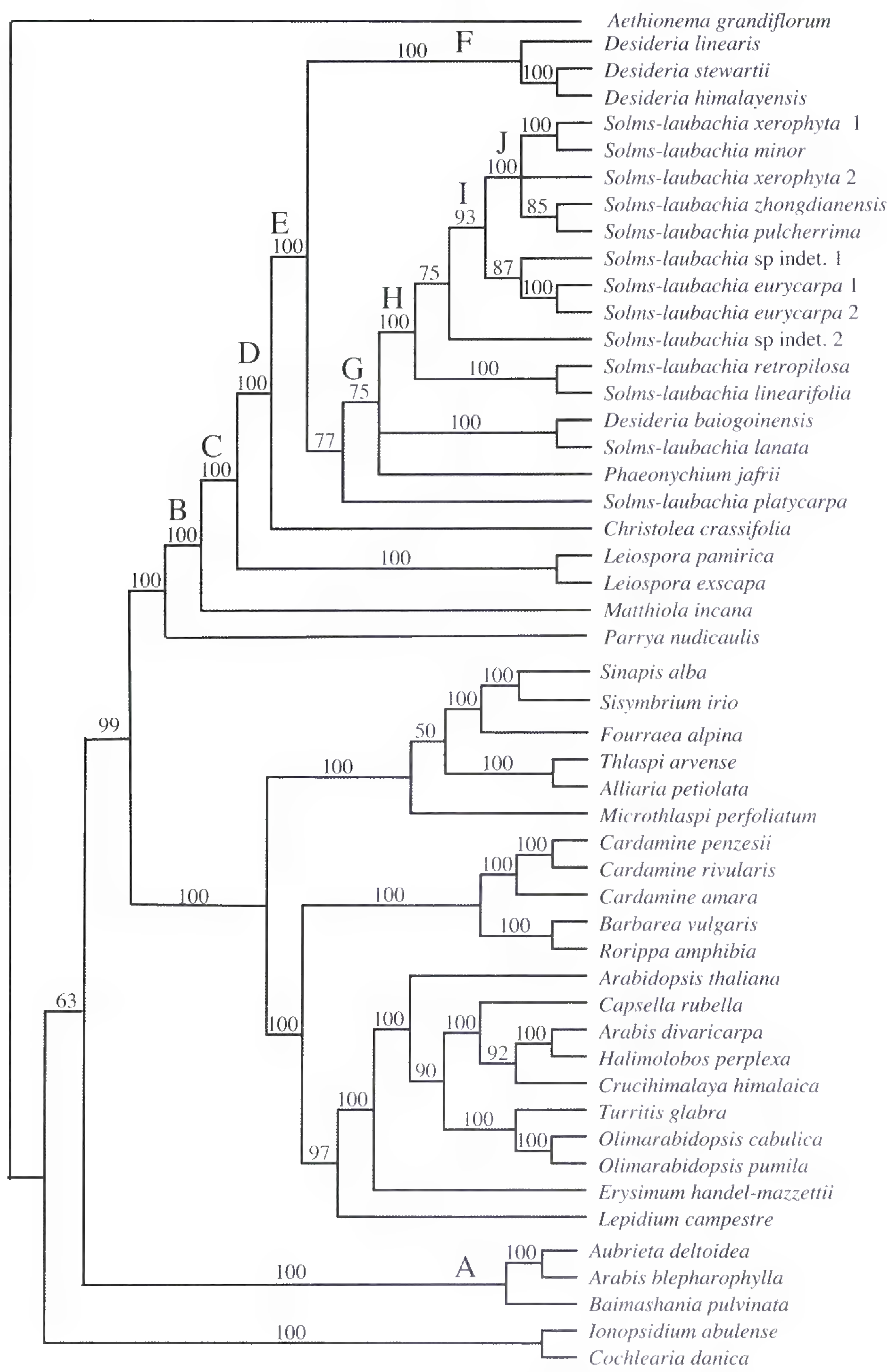


Figure 2. Majority consensus tree inferred from Bayesian analyses of the combined data set of *matK* and *Chs* genes. Numbers at branches are posterior probabilities, and letters A–J refer to clades discussed in the text.

in *Solms-laubachia*, suggesting that *Desideria* may be polyphyletic and *Solms-laubachia* paraphyletic. *Phaeonychium jafrii* is either sister to a weakly supported clade (G, Fig. 1) containing *Solms-laubachia* and *D. baiogoinensis* or is embedded within *Solms-laubachia* (G, Fig. 2). Therefore, our results support an expanded *Solms-laubachia*, i.e., *Solms-laubachia* s.l., including species of *Desideria* and *P.*

jafrii as well. However, *Phaeonychium* is a heterogeneous genus of seven species distributed in central Asia and centered in southwestern China (Al-Shehbaz, 2000a; Zhou et al., 2001). More species are needed to further test the phylogenetic relationships of *Phaeonychium* before a proposal can be made to merge it with *Solms-laubachia* and *Desideria*. Nevertheless, sequences of *matK* and *Chs* suggest the union of *Solms-laubachia* and *Desideria*.

Within *Solms-laubachia*, *S. retropilosa* and *S. linearifolia* are closely related (bootstrap = 74%, Fig. 1, and posterior probability = 100%, Fig. 2). *Solms-laubachia minor*, *S. pulcherrima*, *S. zhongdianensis*, and two accessions of *S. xerophyta* form a clade (bootstrap = 66%, Fig. 1, and posterior probability = 100%, Fig. 2). However, two accessions of *S. xerophyta* do not cluster together, with one (*Yue 0250*) from Sichuan and the other (*Yue 0251*) from Yunnan. Further morphological studies are needed to determine whether they might represent different species. Given the low phylogenetic resolution from *matK* and *Chs* data for interspecific relationships of *Solms-laubachia* s.l., further studies with additional data from more rapidly evolving DNA regions are warranted.

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